**Staphylococcus aureus** mediastinitis and sternal osteomyelitis following median sternotomy in a rat model

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Objectives: Median sternotomy (MS) wound infections are severe complications causing high morbidity and mortality after cardiac surgery. We aimed to develop a new *Staphylococcus aureus* mediastinitis and sternal osteomyelitis model in rats that can be used to evaluate the efficacy of new antimicrobial treatments.

Methods and results: A complete MS wound was induced in anaesthetized rats. *S. aureus* was injected into the sternum. Kinetics of bacterial growth in the sternum (10⁷ cfu/sternum) was assessed for histopathology and bacterial counts. A non-infected MS group served as a control. To evaluate antibiotic efficacy, 5 days of intraperitoneal vancomycin therapy (50 mg/kg, twice a day) was initiated 24 h following bacterial challenge. Macroscopic and histological examination confirmed that infection resulted in sternitis and mediastinitis. *S. aureus* bacterial counts in the sternum were inoculum-dependent, and it was proven that infecting rats with an inoculum of 10⁷ cfu/sternum induced mediastinitis and sternal osteomyelitis. At this inoculum, bacterial counts in the infected sternum increased with time, reaching a maximum level of 2 ± 1 × 10⁷ cfu/g of sternum 8–12 days post-infection and then decreased with time to 2 × 10⁴ cfu/g of sternum 20 days after infection. Histological changes paralleled bacterial counts. Vancomycin administration showed a protective effect against induction of sternal osteomyelitis; sternums from vancomycin-treated rats showed a significant decrease in *S. aureus* counts by 0.72 ± 0.35 log cfu/g compared with untreated controls (P = 0.0162).

Conclusions: This new rat model of *S. aureus* sternal osteomyelitis and mediastinitis allows quantitative measurement of bacterial counts in the sternum. This model is reproducible and simple and thus suitable for the evaluation of new antimicrobials and new treatment modalities in MS infections.

Keywords: experimental model, infection, vancomycin, *S. aureus*, antibiotic treatment

Introduction

Deep sternal wound infections pose a serious problem in cardiac surgery despite continuing efforts to improve perioperative conditions. Mediastinitis and sternal osteomyelitis remain the most dreaded complications after median sternotomy (MS) incisions and were reported to occur in 0.5% to 8% of patients undergoing cardiac surgery.¹–⁶ These complications are often associated with significant morbidity and mortality rates of up to 45%,¹–⁶ with prolonged hospitalization³,⁴ and additional surgical procedures, as well as prolonged antibiotic therapy and its inherent high costs.⁵,⁶ Most MS wound infections are related to the host or to various technical factors and less to infectious outbreaks, with both endogenous and exogenous pathways having been found to play a role in their occurrence.⁷ *Staphylococcus aureus* is the most common pathogen isolated from MS wound infections after cardiac surgery as well as from bacteraemic blood cultures.⁸–¹¹ Epidemiological studies point to an increasing trend in antibiotic resistance, with the appearance of progressively more cases of methicillin-resistant *S. aureus* (MRSA) strain infections, causing an increase in mortality, duration of hospitalization and hospital charges.⁷,¹⁰–¹³ Moreover, an ageing and more morbid cardiac surgery patient population further increases the incidence and severity of MS wound infections.¹⁰–¹³ Thus, new antimicrobials or treatment modalities with increased efficacy are being sought. There is a concomitant need for an animal model that

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can allow the efficacy of such treatments to be tested and that can help to develop protection against post-operative mediastinitis and sternal osteomyelitis. There are a number of experimental animal models found in the literature for sternal osteomyelitis or mediastinitis. Only a few of them include MS incision in rats or employ S. aureus infection as an integral part of the model. We present here a new experimental rat model that studies the natural history of S. aureus infection following an MS wound involving sternal osteomyelitis and mediastinitis in rats, and prove the effect of a systemic antibacterial as treatment against this infection. Practical employment of the model in evaluating protection against S. aureus osteomyelitis was tested with vancomycin and was found to be suitable for evaluating new antimicrobials and therapy strategies for S. aureus infections. The ultimate aim is to reduce morbidity and mortality rates of infection after cardiac surgery.

Materials and methods

Animals and experimental conditions

Male rats, 8 weeks of age, weighing 250–270 g (Harlan Laboratories, Rehovot, Israel) were housed one rat per cage in a central animal research facility. The facility maintains an environment of controlled temperature and relative humidity, with a 12 h light/dark cycle. Rats were supplied with sterile bedding, standard food and water, and were acclimatized for 10 days before each experiment. Acetaminophen (0.25 mg/mL) in the drinking water was used as a post-operative analgesic. The animals were individually labelled and weighed prior to each experiment, and were sacrificed by carbon dioxide at its termination. All procedures, care and handling of the animals were reviewed and approved by the Institutional Animal Care and Use Committee at the Tel-Aviv Sourasky Medical Center.

MS model

Prior to surgery, the rats were anaesthetized by an intraperitoneal injection (0.5 mL) of a mixture of ketamine (90 mg/kg) and xylazine (10 mg/kg). The hair on their chest was thoroughly removed by a razor. Each animal was secured in a supine position and the chest was clipped and prepared for the operation with povidone–iodine and ethyl alcohol. Following a midline skin incision, both pectoralis major muscles were dissected and divided on the medial border from the insertion to the sternum, leaving the sternal bone well exposed. An MS incision was performed using a number 15 blade, leaving part of the narrow sternum on both sides. Metastatic haemostasis was achieved using sterile sponges.

Bacterial strain and infection

A clinical isolate of S. aureus (strain 18454) isolated from an infected sternum of a patient with sternal osteomyelitis was used for all experiments. The strain was susceptible to all standard antistaphylococcal antibiotics tested by a standard susceptibility testing method recommended by the CLSI. Infecting inoculum was freshly prepared before each experiment, and the bacterial titre was confirmed by quantitative cultures. Infection was induced immediately after MS incision by a precise injection of 0.05 mL of a logarithmic culture of S. aureus into the exposed sternal edges and the spongiosa. The muscle layer and skin were carefully sutured in layers with 4-0 nylon monofilaments. No external bandage for wound protection was applied.

Monitoring and evaluation

The rats were returned to their cages after MS incision and bacterial injection, and observed until they had fully recuperated from the anaesthesia. The experiments were typically terminated 30 days after inoculation of bacteria. The animals’ survival was assessed daily, and the chest wounds were examined daily for induration, oedema, purulence or dehiscence. The appropriate S. aureus infecting dose for inducing osteomyelitis was determined by performing MS incisions on rats (in groups of 5–8) followed by injection of 1 × 10^{3}, 1 × 10^{4}, 1 × 10^{5} or 1 × 10^{6} cfu/sternum. Ten days after MS and infection, the animals were sacrificed and their sternal bone was removed aseptically, weighed and homogenized. Quantitative bacterial counts of homogenates and histopathology examination for osteomyelitis were determined/ performed for each specimen and compared with the uninfected group of animals that had undergone an MS incision.

Sternal S. aureus growth kinetics

Once the optimal infecting inoculum of S. aureus had been chosen, the kinetics of S. aureus bacterial growth in the sternum was studied by determining S. aureus bacterial counts in the sternum at different times after MS infection. Twenty rats with MS incisions were injected with 1 × 10^{7} cfu/sternum. Four rats were sacrificed on post-operative days 2, 5, 8, 12 and 19 after MS wound infection. The chest incision was opened and the sternal area exposed. The sternum was photographed and documented for abscess formation and necrosis, and quantitative bacterial cultures of the sternum and histopathology were performed.

Antibiotic treatment

The infected MS wound was challenged with antibiotic treatment. The 20 rats with MS incision were divided into three groups. The first group (n = 8) included S. aureus-infected (1 × 10^{7} cfu/sternum) MS incisions and treatment with systemic vancomycin, which was given intraperitoneally 50 mg/kg every 12 h for 5 days starting at 24 h after MS incision and infection. The second group (n = 8) included controls with infected MS incision that received placebo injections of phosphate-buffered saline (PBS) according to the same regimen as the vancomycin-treated group. The third group (n = 4) had undergone MS incision with no infection and no treatment. The experiment was performed twice and 12 animals were included in the analysis. Quantitative bacterial cultures of the sternum and histopathology were performed.

Histopathology assessment

Histopathology examination was performed on all sacrificed rats to assess the infection process of the bone and the surrounding soft tissue. The sternum of each animal was removed in a sterile manner and fixed in 10% formaldehyde solution. Cross-sections were taken from the bone, processed for standard haematoxylin and eosin staining and evaluated by light microscopy.

Microbiological analysis

Samples from each animal’s sternum were taken aseptically, weighed, homogenized and resuspended in a sterile saline solution. Bacterial counts expressed as S. aureus/g of sternum were determined by serial dilutions of the sample and by colony counting on brain heart infusion agar plates after an overnight incubation.
The average concentration of *S. aureus* in the sternum was calculated from the average of bacterial counts obtained from rats that were sacrificed at each time point. The effect of vancomycin treatment on *S. aureus* bacterial counts in the sternum was expressed as the median cfu/g of sternum bone for each group.

**Statistical analysis**

Statistics were run in Stata version 7 (Stata Corp., College Station, TX, USA). The relationship between the size of *S. aureus* bacterial inoculum injected and bacterial counts from the sternum, during establishment of the model, was quantified using Spearman correlation. The differences in bacterial counts between the vancomycin-treated rat groups and the saline-treated rat groups were analysed using Mann–Whitney’s non-parametric test. Differences were considered to be statistically significant with a *P* value of 0.05 or less.

**Results**

Rats included in this study survived MS incision followed by infection with *S. aureus* using infecting inocula ranging from $10^5$ to $10^7$ cfu/sternum. Rats receiving an inoculum of $10^4$ cfu/sternum overcame the infection completely 10 days after infection, with no bacteria recovered from the sternum. A high inoculating inoculum of $10^5$ cfu/sternum led to a high mortality rate (four out of five rats). Subjective macroscopic examination 10 days after MS infection revealed an abscess surrounding the sternum with purulent discharge in most of the rats injected with $10^5 – 10^7$ cfu (Figure 1a). Sternal histopathology on day 10 after MS *S. aureus* infection displayed acute osteomyelitis with bacterial colonies and soft tissue abscesses (Figure 1c), when compared with the control group of rats with MS incision and no bacterial challenge. The latter showed an acute and chronic inflammatory process, with no osteomyelitis, bacterial colonies or soft tissue abscesses (Figure 1b).

To determine the effect of the size of infecting inocula, rats were infected with $1 \times 10^4 – 1 \times 10^7$ cfu/sternum. Propagation of *S. aureus* infection in the sternum revealed inoculum-dependent behaviour reflected by sternal bacterial counts and histopathology (Figure 2). There was a direct relationship between the size of the infecting *S. aureus* inoculum from $1 \times 10^5$, $1 \times 10^6$ or $1 \times 10^7$ and the bacterial counts recovered from the sternum 10 days after infection ($3.9 \times 10^5$, $3.6 \times 10^5$ and $2.34 \times 10^7$/g of sternum, respectively, *r* = 0.8 using Spearman correlation, Figure 2). Histopathology examination of the sternum with $1 \times 10^5$ cfu of *S. aureus* per sternum showed scar tissue with chronic inflammatory infiltrates and regenerative changes. A higher inoculum of up to $1 \times 10^7$ cfu of *S. aureus* per sternum resulted in acute inflammatory infiltrates with acute sternal osteomyelitis, bone sequestrum and soft tissue abscess formation. A challenge inoculum of $1 \times 10^7$ *S. aureus* per sternum was chosen for further studies; it was non-lethal, bacteria could be cultured from all the sternum bone preparations and acute osteomyelitis with high bacterial counts was demonstrated in all cases without exception.

Growth kinetics of *S. aureus* after sternal infection with $10^7$ cfu/sternum showed an increase in bacterial counts in the sternum from the second to the eighth day after infection, reaching a peak and then gradually decreasing from day 12 to day 19 post-infection (Figure 3). Vancomycin treatment administered after the MS wound was infected significantly decreased the *S. aureus* counts in the sternum compared with the *S. aureus* counts in the placebo (PBS)-treated rats. Median bacterial counts of *S. aureus* in the sternum bone (lower quartile/upper quartile) were as follows: control group, $1.2 \times 10^7$ cfu/g ($1.5 \times 10^5/7.4 \times 10^7$) and vancomycin group, $4.2 \times 10^4$ cfu/g ($2 \times 10^3/2.7 \times 10^5$) (Figure 4, *P* = 0.01). No bacteria were recovered from sternum specimens removed from the non-infected MS wound control rats. Sternal histopathology of specimens taken on days 8 and 12 after MS *S. aureus* infection displayed acute osteomyelitis with bacterial colonies and soft tissue abscesses in the placebo-treated rats. Vancomycin-treated rats showed acute and chronic inflammation with regenerative changes and no bacterial colonies.

**Discussion**

Post-surgical mediastinitis and sternal osteomyelitis are associated with high morbidity and mortality rates, diminished quality of life and major economic impact. With the expansion of cardiac surgery to include the more severely ill and elderly...
patients, and the growing prevalence of MRSA in tertiary care and community hospitals, more drug-resistant strains are being isolated from post-surgical MS wound patients. Clinical studies have shown that post-surgical mediastinitis and sternal osteomyelitis due to MRSA are particularly costly and deadly and are associated with an 11-fold increase in associated mortality and excess hospital charges.

Numerous MS animal models have previously been described in the literature. These models were designed to study new surgical techniques and treatment adjuvants, aiming to lower post-operative complications. There are, however, few sternitis or mediastinitis models studied on smaller animals (rodents) that were challenged with bacteria. To ensure the development of bone infection, some of these models demonstrated osteomyelitis using a foreign body and studied mediastinitis with no MS incision, but instead with direct mediastinal infection. Both Ozcan et al. and Ogus et al. described the effect of adjuvant therapy on an S. aureus-infected MS wound rat model, but did not relate their results to S. aureus sternal growth kinetics or to sternal histopathology of the infected non-treated group. To the best of our knowledge, no previous article has described the pathophysiology of sternal osteomyelitis and mediastinitis following an MS wound S. aureus infection. The most important requirements of a reliable animal model are an infection rate of 100% without spontaneous curing, low mortality of the experimental animals and small variance between the infected animals within a given experiment.

We present this current rat model that studies both bacterial growth kinetics and histopathology of the sternum and challenges it with systemic antibiotics. In our model, the infecting S. aureus inoculum was non-lethal, and the bacterial growth from the sternum after infection was found to be inoculum-dependent. The infecting load of 1 × 10^7 cfu of S. aureus per sternum resulted in complete recovery from the infection, with no bacterial growth from the sternum. Increasing the bacterial load from 1 × 10^5 to 1 × 10^7 cfu of S. aureus per sternum resulted in increasing sternal bacterial growth. Histopathology studies of the sternal samples were consistent with the bacterial counts and displayed acute osteomyelitis and soft tissue abscesses in proportion to the size of the infecting load. The MS incision by itself did not induce sternal infection, in that control rats that underwent MS incision with no infection had no sternal bacterial growth and no signs of osteomyelitis or soft tissue abscesses. Vancomycin significantly decreased the S. aureus bacterial load in the sternum (P = 0.01). Results presented are the averages of two independent experiments.

An S. aureus growth kinetics study demonstrated maximal bacterial growth on days 8–12 after infection (Figure 3). This was when sternal bacterial growth peaked and so emerged as the optimal time to determine bacterial counts.

Challenging S. aureus-infected MS wounds with the systemic antibiotic vancomycin significantly decreased sternal bacterial counts (Figure 4). Antibiotic treatment was found to be effective not only in reducing sternal bacterial growth but also by
S. aureus sternal osteomyelitis in a rat model

Attenuating the osteomyelitis process demonstrated by histopathology. Placebo treatment (control rats treated with PBS) had no effect on bacterial growth, with the wounds displaying high bacterial counts and acute osteomyelitis with soft tissue abscesses.

This study has some limitations. The anatomy and collateral blood supply to the sternum in rats may be different from that in humans. Furthermore, this model does not take into account the ischaemic damage to the sternum after harvesting both internal mammary arteries.\(^7,20\)

There is no consensus on the optimal management of post-sternotomy mediastinitis, but long-term antibiotic treatment is universally accepted as being fundamental to the treatment process.\(^7,10\) Although antimicrobial treatment should be initiated promptly, there is no agreement either on the choice of the most suitable drug or on the preference for a combination therapy over monotherapy,\(^10\) and new antibacterials are constantly being sought in this era of increasing drug resistance. Our model can provide a framework for studying the efficacy of new antibacterial and treatment modalities in acute mediastinitis and sternal osteomyelitis. Different resistant strains can be challenged with different antibacterials, and various sternal bacterial growths and the histopathology can be studied. This model is simple, relatively inexpensive and reproducible, facilitating comparison of different antibacterial regimens.

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Transparency declarations

None to declare.

Supplementary data

A colour version of Figure 1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References